Peer Community In
Ecology

Indirect effects of parasitism include increased profitability of prey to optimal foragers

Luis Schiesari *based on peer reviews by* ***Thierry DE MEEUS*** *and* ***Eglantine Mathieu-Bégné***

Loïc Prosnier, Nicolas Loeuille, Florence D. Hulot, David Renault, Christophe Piscart, Baptiste Bicocchi, Muriel Deparis, Matthieu Lam, Vincent Médoc (2023) Parasites make hosts more profitable but less available to predators. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Ecology.
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Even though all living organisms are, at the same time, involved in host-parasite interactions and embedded in complex food webs, the indirect effects of parasitism are only beginning to be unveiled.

Prosnier et al. investigated the direct and indirect effects of parasitism making use of a very interesting biological system comprising the freshwater zooplankton *Daphnia magna* and its highly specific parasite, the iridovirus DIV-1 (Daphnia-iridescent virus 1). *Daphnia* are typically semitransparent, but once infected develop a white phenotype with a characteristic iridescent shine due to the enlargement of white fat cells.

In a combination of infection trials and comparison of white and non-white phenotypes collected in natural ponds, the authors demonstrated increased mortality and reduced lifetime fitness in infected *Daphnia*. Furthermore, white phenotypes had lower mobility, increased reflectance, larger body sizes and higher protein content than non-white phenotypes. As a consequence, total energy content was effectively doubled in white *Daphnia* when compared to non-white broodless *Daphnia*.

Next the authors conducted foraging trials with *Daphnia* predators *Notonecta* (the backswimmer) and *Phoxinus* (the European minnow). Focusing on *Notonecta*, unchanged search time and increased handling time were more than compensated by the increased energy content of white *Daphnia*. White *Daphnia* were 24% more profitable and consistently preferred by *Notonecta*, as the optimal foraging theory would predict. The authors argue that menu decisions of optimal foragers in the field might be different, however, as the prevalence – and therefore availability - of white phenotypes in natural populations is very low.

The study therefore contributes to our understanding of the trophic context of parasitism. One shortcoming of the study is that the authors rely exclusively on phenotypic signs for determining infection. On their side, DIV-1 is currently known to be highly specific to *Daphnia*, their study site is well within DIV-1 distributional range, and the symptoms of infection are very conspicuous. Furthermore, the infection trial – in which non-white *Daphnia* were exposed to white *Daphnia* homogenates - effectively caused several lethal and sublethal effects associated with DIV-1 infection, including iridescence. However, the infection trial also demonstrated that part of the exposed individuals developed intermediate traits while still keeping the non-white, non-iridescent phenotype. Thus, there may be more subtleties to the association of DIV-1 infection of *Daphnia* with ecological and evolutionary consequences, such as costs to resistance or covert infection, that the authors acknowledge, and that would be benefitted by coupling experimental and observational studies with the determination of actual infection and viral loads.

References:

Prosnier L., N. Loeuille, F.D. Hulot, D. Renault, C. Piscart, B. Bicocchi, M, Deparis, M. Lam, & V. Médoc. (2023). Parasites make hosts more profitable but less available to predators. BioRxiv, ver. 4 peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2022.02.08.479552

Reviews

Evaluation round #2

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Version of the preprint: 3

Authors' reply, 23 June 2023

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Decision by Luis Schiesari, posted 29 March 2023, validated 30 March 2023

Dear Dr Prosnier

Thank you for your revised version of ´Parasites make hosts more profitable but less available to predators´.

Both reviewers and myself consider that your manuscript is now improved, in particular with respect to (i) the clarification of the Methods (Table 1, for example, is helpful) and (ii) the reorganization of the material presented in the Results and Appendices, which (iii) increased the emphasis on the joint analysis (MFA) of results of the experimental infection.

However, there are still several points made by the referees and myself that have to be considered prior to recommendation of your manuscript by PCI Ecology.

From my side, I am still concerned about the reliance on phenotypic analysis for determination of infection status, and I do not think the rebuttal letter did a sufficiently thorough job in addressing my concerns.

The rebuttal letter is clear in explaining that Daphnia were never actually tested for infection status.

But I also asked whether the authors could instead provide reflectance data for Daphnia that were exposed to presumably infected Daphnia cadavers (i.e., because had the white phenotype) versus Daphnia that were exposed to presumably uninfected Daphnia cadavers (i.e. because had the non-white phenotype). This would be easier for the readers to accept than presenting reflectance data from wild individuals, as the experimental results were indeed consistent with infection of at least part of the individuals.

I then asked whether Daphnia from Bercy and La Villette had previously been subject to DIV-1 testing. If a previous study did demonstrate that Daphnia and DIV-1 actually coexist in these two ponds, the readers would feel more comfortable about your studies based on wild caught Daphnia. If no one ever tested Daphnia in these two ponds for DIV-1, the readers would feel a little more comfortable if the authors were able to say ´DIV-1 infection of Daphnia magna is common in ponds surrounding Paris´ (with the appropriate references) or ´DIV-1 is a virus parasite of Daphnia magna that is widespread in ponds in Central Europe, and iridescence in D. magna cannot be attributed to any other parasite or physiological change to date´ (with the appropriate references).

I did not receive any clear response to these two queries.

On the same line, the authors need to carefully go through the manuscript and adjust the text regarding the infection status of wild caught Daphnia. For example, lines 340-342 read ´Concerning D. magna coloration (Measure 6), we found three peaks in the spectrum that were compared between healthy and infected individuals using Wilcoxon signed-rank tests, because data were not normally distributed´. Likewise, the legend in Figure reads ´Effects of DIV-1 on reflectance between 280 and 850 nm. Blue (dashed) lines are healthy D. magna and red (solid) lines are infected D. magna.´

In no case you can say that wild Daphnia are infected or uninfected, or healthy or infected. Perhaps you could say ´presumably infected´ and ´presumably uninfected´, or ´white phenotype´ and ´non-white phenotype´ in every case that you refer to wild Daphnia. Of course it does not read as nice, but this is really all you can say.

I am looking forward a revised version of your manuscript, as well as a rebuttal letter to the comments raised by the two referees and myself.

Sincerely, Luis Schiesari

Reviewed by Thierry DE MEEUS, 06 March 2023

Comments on the 2nd version of the preprint entitled "Parasites make hosts more profitable but less available to predators" by Prosnier et al. submitted to PCI Ecology

Thierry de Meeûs

I read the second version of this manuscript with interest and still think it deserves being recommended by PCI Ecology.

With a diversified collection of sophisticated experimental and analytical tools, Prosnier et al studied the interaction between a virus, DIV-1, its host (Daphnia) and Daphnia predators (backswimmer insects, and European minnow fishes). They demonstrated that infections with this virus, which only infect Daphnia and not its predators, produced an ecological syndrome in the affected food webs. Infected Daphnia are more profitable to predators (21% energy increase), and suffer from higher mortalities. Interactions between the virus and the host shaped different extended phenotypes, from more visible bigger and more profitable preys, to less visible (resistant) individuals but with lower lifespan and a lower mobility as compared to healthy (unexposed) individuals. These results represent important advances in the understanding of food web functioning and the part played by infectious agents.

Despite the undisputable quality of this work, I believe that there are still some minor problems that will require being addressed before this preprint can be recommended.

Rebuttal letter to my remarks

I) About the threshold of 5%. I still feel quite uncomfortable with this. Everybody knows that the conventional threshold is 5%. However, not everybody realize that this is a convention, and that each researcher is responsible for the statistical decision to make with a p-value=0.04999, or 0.0501. This convention, by definition, is not an undisputable law. I still think that the concerned sentence is useless.

II) About parametric/non parametric tests. Authors replied ”We did the non-parametric test (Kruskal Wallis/Wilcoxon) and found results that are consistent with the ones we present here...After discussion with statisticians, we kept the parametric tests with log transformation, rather than a non-parametric test, because it is recognized that rank tests lead to a “loss of information”, thus are “less efficient or less powerful”; consequently “non-parametric methods are justified when conditions are not satisfied for other methods, after variable transformations” (Dagnelie, 2006, Statistique théorique et appliquée, 2nd ed., de Boeck)”.

This is an odd answer. 1) If the non-parametric tests gave the same results, I do not see where the ”loss of information” is. 2) If the log transformed data are not normally distributed and without homoscedasticity, then the result may be not so good. I could not find where the authors tested for the normality and homoscedasticity of their log-transformed data. 3) In my long carrier, dealing with parasite distributions and other non-normal data, I have met several situations when the statistical analysis undertaken with non-parametric statistics gave a significant result, while the parametric test did not. So, the ”loss of information” is not always on the same side.

If the non-parametric test provided a non-significant result, while the parametric one outputted a significant one with log transformed data, I would indeed recognize that further argument would have been necessary to explain why. However, the results was apparently the same. So why bother?

III) About random effect. I am not convinced by the argument of the authors. What they describe in their rebuttal corresponds more to a nested factor than to a random one. I am not a statistician, but I am not sure that a nested factor would have provided the same result as the mixed model used by the author.

IV) About one-sided tests, authors wrote: ”It seems not technically possible to do a one-sided test for survival analysis (and it should not affect our conclusions)”.

One little trick of mine when a software does not provide one-sided tests is to check the direction of the response and to halve the p-value if the response is in the expected direction, and compute 1-(p-value/2) otherwise. It should provides an approximate one-sided p-value. This is really a minor remark.

Amended text (most are minor to very minor remarks, but see point 6 please)

1) Line 106: May be authors could mention here that Daphnia iridescent virus 1 only infects Daphnia and not its predators.

2) Line 313: I would have undertaken a log normal regression (glm with poisson), without log transformation instead of an anova on log transformed data.

3) Line 334: If Holm adjustment method is the sequential Bonferroni, as I think it is, then I would suggest using the less conservative Benjamini and Hochberg (BH in R).

4) Lines 351-353: ”Based on the data obtained (Measures 5 and 7), 100 healthy and 100 infected D. magma were generated using a bootstrapped method (5,000 iterations), allowing for each individual to calculate a profitability.”

Please rephrase, e.g.:

”Based on the data obtained (Measures 5 and 7), 100 healthy and 100 infected D. magma were generated using a bootstrapped method (5,000 iterations). This procedure allowed computing a profitability for each Daphnia individual.”

5) Line 357: I think that, if I understood well what it is about, to directly test if the distribution of your p-values significantly deviates from what would be expected if each test had been undertaken under H0, you

can undertake a generalized binomial procedure (Teriokhin et al., 2007), with MultiTest (De Meeûs et al., 2009). Nevertheless, combined tests need being independent and testing the exact same H0. I tried to find it in the R supplementary files to get the series of p-values to combine, so that I could undertake it myself, but failed to find those (it probably would not change much things). Where are these data and the associated Kolmogorov-Smirnov test (KS)?

6) Line 419: Following the previous point, Table C8 is missing. This, with the missing KS test and the series of p-values, represent an important remark regarding the policy of PCIs.

References

De Meeûs, T., Guégan, J.F., Teriokhin, A.T., 2009. MultiTest V.1.2, a program to binomially combine independent tests and performance comparison with other related methods on proportional data. BMC Bioinformatics 10, 443.

Teriokhin, A.T., De Meeûs, T., Guegan, J.F., 2007. On the power of some binomial modifications of the Bonferroni multiple test. Zh. Obshch. Biol. 68, 332-340.

Reviewed by Eglantine Mathieu-Bégné, 20 March 2023

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Evaluation round #1

DOI or URL of the preprint: `https://doi.org/10.1101/2022.02.08.479552`
Version of the preprint: 2

Authors' reply, 28 February 2023

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Decision by Luis Schiesari, posted 24 July 2022

major review needed

Dear Dr Prosnier,

I have now received feedback from two reviewers regarding your manuscript, ´Parasites make hosts more profitable but less available to predators´.

Both reviewers and myself agree that your manuscript deals with a very interesting general question in ecology, namely, the direct and indirect effects of parasites in a food web context; that the coupling of experimental and observational approaches is a positive aspect of your research; and that there is a combination of relevant response variables being measured.

At the same time, both reviewers and myself understand that your manuscript requires revisions before it can be recommended by PCI. Please see the reviewers´ comments below.

In additipn to the comments raised by the referees, I am concerned that infection was never actually measured. The authors argue that the phenotypic consequence of infection (=iridescence) is well known, and this may be acceptable for part of the response variables (in particular for those that were derived from experimental infection). However, at least in one case the association between phenotype and the assumption of infection is problematic – this is the measure of reflectance (measure 8). That is, measuring reflection as a response to infection when infected versus non-infected individuals were classified based on reflectance is... a circular reasoning. These were wild Daphnia and there was no test for DIV-1. Can you provide reflectance of the experimentally infected Daphnia? Furthermore, the reader would be less concerned if you were able to mention that Daphnia in these particular water bodies were previously tested for DIV-1.

Overall, then, the manuscript is of interest but the authors should undertake a major review, and provide a detailed response letter, in light of the comments given by the referees and myself before it can be Recommended by PCI.

Best regards, Luis Schiesari

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Additional minor comments by the Recommender

Line 53. ´concurrently in addition´ is redundant.

Lines 200-202. Body size was measured in naturally infected individuals, but also in naturally non-infected individuals. So it would be best to use a different term (maybe ´wild Daphnia´?) in this as well as elsewhere in the manuscript (e.g. line 210)

Lines 261, 265. ´proposed´ reads odd. Maybe ´offered´?

Line 279. Lower case i, instead of capital I

Lines 438-440. This hypothesis was not formulated before, nor there is a citation for such a hypothesis being proposed in the literature. Or, alternatively, frame it in a way that it makes clear that this is your interpretation, a posteriori.

Lines 444-446. Unclear. Are you proposing lower speed is a cause for larger size?

In the Figures, Legends for the colors (i.e. colored box-plots and lines) are usually missing, and in the Results please reinforce the nature of the results (that is, make sure that the reader follows whether this or that effect is based on experimental or observational evidence).

Figure 1. Not clear how survival was measured and analyzed. If all newborns were exposed at the same time, why to break down the mortality of those that died before brooding and those that died after brooding? Should we assume that the blue continuous line and the red intermittent line in Figure 1A are cumulative, i.e., they comprise all individuals entering the experiment?

Reviewed by Thierry DE MEEUS, 23 June 2022

Comments on the preprint entitled "Parasites make hosts more profitable but less available to predators", by Prosnier et al, and submitted to PCI Ecology

The preprint relates an interesting study on several aspect of the ecology of a complex host-parasite-predator system.

I read the material and methods with interest, but realized that I was unfamiliar with all techniques used. I thus cannot assess the validity and quality of such experiments and measurements and trusted the authors about the reliability of those.

There are globally too many analyses that study the same things (redundancy). Authors should get rid of the less significant ones and stick to the most important analyses and results. I also think that some statistical analyses deserves being redone.

I believe this preprint is worth being recommended, providing the authors undertake important modifications. Authors will find below different points raised during my reading of the manuscript.

1) Lines 281-282: The levels of significance announced at 5% in the M&M section looks a little old-fashioned to me. Depending on sample sizes, on what is worse between rejecting or accepting H0, and on the number of tests handled, common researchers may adjust levels of significance. Please delete.

2) Line 294: Is there not a better way to analyze these data but to log transform those? Did the author checked if Kruskal Wallis provided results that proved consistent with the Anova? Could you not use a glm model with customized error? Transforming data is far from ideal.

3) Line 312: I do not know about dates, but I do not think pound can be considered as a random effect. I indeed hardly believe that these two pounds share exactly the same physico-chemical, and ecological parameters. Several results confirm that there is a non-random effect of pounds.

4) Line 314: Please rewrite ”analyze” into ”analysis”.

5) Line 348: We expect that exposed (or infected) individuals produce less offspring, and hence tests should be one-sided. Was this the case? If not then the test may be significant after all. This applies to all comparisons between uninfected, exposed and infected Daphnia.

6) Lines 387-392: I think Figure C3 is interesting, very meaningful, and should be in the main text. Too many obscure quantities are given here. Cannot you just say that, according to the figure, it is obvious that, between 300 and 800 nm, infected Daphnia reflect much more light than healthy ones for most of the spectrum, except for two very narrow wavelengths intervals (p-value<0.001)?

7) Lines 395-410: The legend indicating the identity of different colors should be represented in the Figure 4. I am surprised that search times difference was not significant. I would redo this test with a one sided signed rank Wilcoxon test for paired data on a single table combining 1st, 2nd and 3rd preys.

8) Lines 424-434: I do not understand this. Infected hosts produced significantly less clutches of similar size as compared to healthy ones, so fecundity is negatively affected by the virus. Authors should avoid multiple and contradictory sentences in the discussion and just go straight to the major points.

9) Lines 443-444: Same as point 8. Fecundity is the natural capability to produce offspring. To this respect, this virus has a negative effect on the fecundity of Daphnia. Alternatively, maybe I missed something and this needs being rephrased.

10) Line 496: I do not understand why a decrease in Daphnia populations should necessarily yields a decrease in the fitness of notonects or fishes, unless Daphnia are the only prey for such predators, which I seriously doubt.

11) References list: Please harmonize the format. Many article titles display a capital for the first letter of each word, which is not standard.

12) Appendix A: I understand that the t-test was paired by individual hosts, but I have several concerned. Why did authors log transform the data? I would instead advise undertaking a Wilcoxon signed rank test for paired data on untransformed measures. We expect that search time is smaller for infected prey, so I would expect one-sided tests. Was it the case? I could not find p-values. I would redo all these tests with a one-sided Wilcoxon test for paired data, and would analyze a single table with all preys to get a single test (much more powerful).

13) Appendix B is too hard to read, especially to those not familiar with MFA. If first and 2nd axes are explained by a few variables, these should be identified in the graph with the direction of increase. I personally could not interpret these graphics the same as the authors did in the text of this appendix. I noticed several counter-intuitive observations: Life span and size are negatively correlated; NbEgg is negatively correlated with size; mobility is positively correlated with clutch size. Regarding the virus, if I understand well, infected hosts display a larger body size, a smaller life span, and larger clutch sizes (why?); Control are less mobile than exposed hosts (why?). The why needs beuing discussed more clearly for each of these observations.

14) Figure 1C: There is an obvious superimposed effect of infection on survival of hosts, which interfere with the results presented here. A better comparison would be the absolute total numbers of eggs produced by healthy and infected hosts.

15) Raw data sets: There are no legends in the datasets. Legends should be added at least in excel files.

16) I am not sure that Notonecta usually use D. magna as preys, as they may prefer larger preys, so maybe this would require at least a little sentence of discussion.

17) There is an important question that is not discussed in the manuscript. Is the DIV1 virus staying in or infecting the Notonecta? If yes, then the increased predation of D. magna and other crustacean (amphipods, isopods), will increase the concentration of viruses in the aquatic environment and maybe the infection of Daphnia.

Reviewed by Eglantine Mathieu-Bégné, 14 June 2022

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